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Angular sensitivity of blowfly photoreceptors: broadening by artificial electrical coupling

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Summary. 1. Electrical coupling between R1-6 photoreceptors was investigated by measuring angular sensitivities and quantum bumps.

2. Recordings were made from two extreme types of cells: Type a: cells with a diffraction-like angular sensitivity profile (see Smakman et al. 1984). Only large bumps could be obtained from these cells. Type b: cells with large, asymmetrical sidebands in the angular sensitivity profile. Large and small bumps could be recorded from these cells, specifically with off-axis illumination.

3. The position of the small sidebands of the type a cells depends strongly on wavelength, as expected for a diffraction curve. The position of the large asymmetrical sidebands in the angular sensitivity profile of the type b cells was found to be independent of wavelength, indicating that these sidebands are not caused by the diffraction pattern of the facet lens.

4. The angular position of the large asymmetrical sidebands corresponds with the position of neighbouring photoreceptors, suggesting electrical interactions between R1–6 photoreceptors.

5. The dependence of this electrical coupling on microelectrode properties was investigated. It was possible to change the degree of electrical coupling by selection of electrodes.

6. The difference in properties of the two cell types encountered are interpreted as an indication that some R1-6 photoreceptors are artificially electrically coupled while others are not. The correlation of the electrical coupling with electrode types and the possible artificial origins of coupling are discussed.

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Introduction

Interactions between retinular cells of invertebrates have been the subject of a number of studies in the recent past, especially in invertebrates with a fused rhabdom (Behrens and Wulff 1965; Shaw 1967, 1969; Lillywhite 1978; Menzel and Blakers 1976; Horridge et al. 1983). More recently interactions between photoreceptor cells in flies, with an open rhabdom, have also been reported (Mimura 1978; Dubs et al. 1981). This suggests that electrical interactions between neighbouring photoreceptors are a widespread phenomenon in invertebrates (see further the reviews of Shaw and Stowe 1982 and Järvilehto 1985).

In flies electrical couplings were inferred from electrophysiological measurements of angular sensitivities of photoreceptors (Mimura 1981; Dubs 1982). Apparently then, the receptive field of a single cell originates from the outputs of a number of different cells, as experiments yielded receptive fields of single cells much broader than expected from the optical properties of the optical elements involved, namely the facet lens and the rhabdomere (see for example Pask and Barrell 1980; van Hateren 1986).

Measuring bump sizes is another electrophysiological means of discriminating between the different signals that contribute to a receptive field. Just as in the locust (Lillywhite 1978), large (*l*) and small (*s*) bumps have been recorded from fly photoreceptors. The l-bumps are evoked by on-axis illumination and the s-bumps by off-axis illumination. This indicates that an l-bump results from photon capture by the penetrated cell and an s-bump from a neighbouring photoreceptor (Dubs et al. 1981).

This hypothesis of electrical coupling in the fly retina thus entails that photoreceptor signals are already summed before they reach the lamina. This can be advantageous for an increase of the signal/noise discrimination at low light levels (for discussion see Srinivasan et al. 1982).

However, the resulting spatial summation means loss of spatial acuity. Therefore, an obvious question arises, namely, why this first step in spatial summation of information already takes place in the retina and not later, for example in the lamina or medulla.

This question is quite pertinent because there is also a system which pools photoreceptor signals. According to Kirschfeld (1967), each large monopolar cell (LMC) in the lamina receives input from six photoreceptors. These photoreceptors are located in different neighbouring ommatidia, in a precise pattern so that they share a common visual axis thus building a so-called 'neuro-ommatidium'. The result is that the LMC is the site for pooling optical inputs coming from one direction, through six different facet lenses. This arrangement yields an increased photon catch, resulting in an improved signal-to-noise ratio, without a deterioration of the spatial acuity (van Hateren 1986). We note that photoreceptors belonging to the same neuro-ommatidium are electrically coupled (Shaw 1984; van Hateren 1986). As these cells share the same visual axis, this coupling is not seen in the spatial sensitivity. However, electrical coupling between photoreceptors within an ommatidium, if one is present, will affect the spatial sensitivity. The latter type of electrical coupling is examined here.

In the LMC, optical inputs coming from one direction are pooled, which raises another question: why is such a pooling system located behind electrically coupled photoreceptors? It seems paradoxical that a pooling system which maintains spatial acuity is positioned behind a spatial summation station (i.e. the retina) where part of the visual acuity is already lost. This point of view provoked us to have a critical look at the methods used by the various authors in measuring the electrical coupling between photoreceptors. It appeared that all had used electrophysiological methods; meanwhile no clear histological indications for electrical interactions between photoreceptors within one and the same ommatidium have been found [see also the discussions of Dubs (1982) and Shaw (1984)].

Moreover, measurements in the bee with non-invasive optical techniques failed to confirm the electrical interactions measured earlier with electrophysiological methods (Bernard and Wehner 1980).

In this report we study the electrical coupling between R1–6 photoreceptor cells within the same ommatidium of the blowfly *Calliphora erythrocephala*. These couplings are described in the first section of the results. It is shown in the second section that measurements of the electrical coupling may be a tool to test the reliability of conventional electrophysiological methods.

Materials and methods

The methods employed were identical to those of Smakman et al. (1984). Briefly, the method of measuring angular sensitivities is as follows.

Electrodes. Glass microelectrodes were made on our laboratory-made Brown and Flaming electrode puller (Brown and Flaming 1977). The tip of the electrode was examined under a light microscope (Zeiss, darkfield, epi-illumination). The 3 *M* KAc-filled electrodes had a resistance of 150–200 MΩ, measured in Ringer's solution.

Angular sensitivity measurements. After preparation, the fly was mounted in the centre of a goniometer platform and a glass microelectrode was lowered vertically through a hole, made in the dorsal part of the cornea. Recordings were made in the daytime from photoreceptor cells in the frontal part of the right eye of female blowflies. Only peripheral photoreceptors R1–6, classified from their spectral sensitivity, were investigated. In most cases the integrity of the optics of the fly's eye was checked using the deep pseudopupil.

After successful penetration of a cell the goniometer platform with fly and intracellular microelectrode was adjusted to a point-light source for maximum response. This point-light source was a flexible lightguide coupled to a motor-driven perimeter. The aperture of the lightguide was 0.2° as seen by the fly. The angular sensitivity profile was measured by moving the lightguide in either the horizontal or vertical plane through the visual field of the cell. The intracellularly recorded light-response of the cell was clamped to a constant value (6 mV, and in some recordings 3 mV) by an analog-digital feedback system in which a neutral density wedge automatically controlled the intensity of the stimulating beam, when the angle of incidence of the stimulating beam varied (Smakman and Pijpker 1983). The position of the density wedge was plotted against the angle of incidence of the stimulating beam yielding the angular sensitivity profile.

Quantum bump measurements. In order to compare the bump sizes measured at the peak of the angular sensitivity function and that of a sideband (Fig. 3A), the setting of the neutral density was first adjusted so that the light response of the penetrated cell in both positions of the light beam was equal to 3 mV, i.e. the reference signal of the feedback system. Subsequently, in both cases the same set of grey filters was added to the beam. After 10 min dark adaptation, bumps were recorded.

Results

The angular sensitivity profiles measured in blowfly photoreceptors often varied considerably in shape. A few examples of spatial sensitivities are presented in Fig. 1. The wavelength used in these

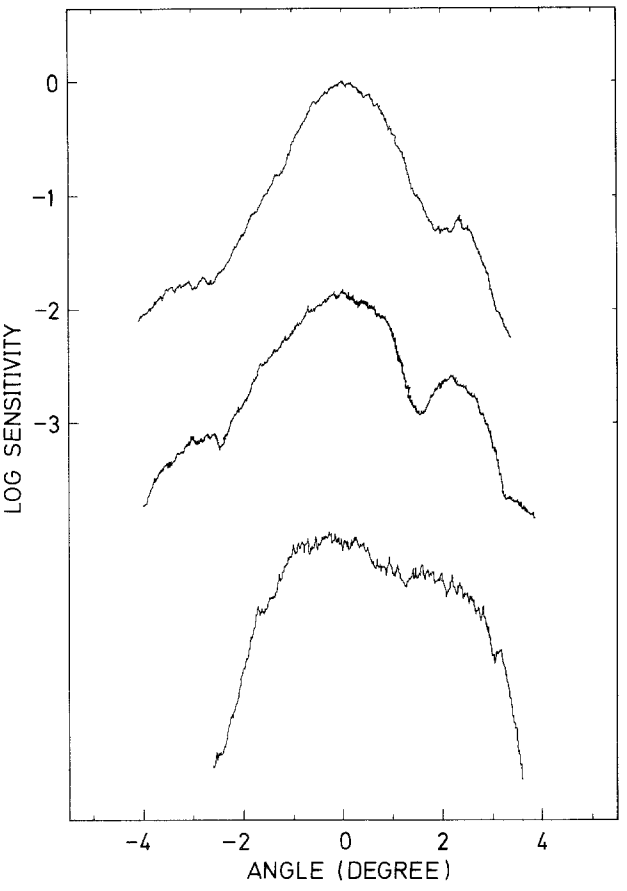

Fig. 1. Angular sensitivities of three different cells, measured at a wavelength of 494 nm. The response of the cell was clamped by the feedback system on a level of 6 mV. Note the difference in amplitude of the asymmetrical sidebands

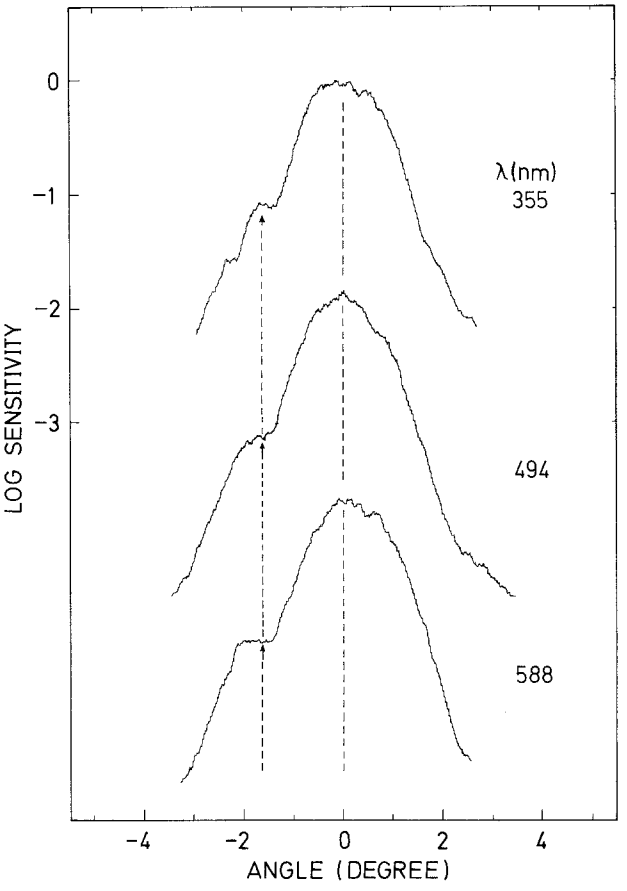

Fig. 2. Angular sensitivities of a single cell measured at wavelengths of 355, 494 and 588 nm. The angular position of the asymmetrical sidebands is independent of the wavelength of the stimulus

measurements was 494 nm. The spatial sensitivity profiles show rather large sidebands which are far from symmetrically positioned on the sides of the profile. The amplitude of these sidebands can vary between 5% to 100% of the peak sensitivity of the penetrated cell, measured on-axis.

There is a close resemblance between these angular sensitivities and the receptive fields of the R1–6 cells of the fleshfly *Boettcherisca peregrina* as measured by Mimura (1981). Mimura concluded that these receptive fields were composed of outputs from different cells. As a first test for this view, namely that sidebands in the blowfly also can originate from interactions between retinular cells, we measured the angular sensitivities of cells with asymmetrical sidebands at different wavelengths (Fig. 2). As can be seen from Fig. 2, the position of the sidebands does not vary noticeably with the wavelength of the stimulus, in contrast to a diffraction pattern (see Smakman et al. 1984). However, the angular separation between peak and sideband is 1.8°, corresponding with the interommatidial angle in the investigated part of the eye (Stavenga, unpublished results).

Large and small bumps

The possibility of electrical coupling between neighbouring photoreceptors was further investigated by measuring bump sizes at different angular positions of the light beam. As noted by Lillywhite (1978), we can expect two populations of bumps from electrical coupling: large bumps, originating from single photons that are captured in the penetrated cell (l-bumps) and small bumps (s-bumps) originating from photons captured in a neighbouring photoreceptor.

The electrical signals generated in the latter case are supposed to leak, via a small resistance barrier, to the penetrated cell.

Before the recordings shown in Fig. 3B, the light beam was positioned at the peak of the angular sensitivity of the cell, thus giving a 3 mV depolarization. Subsequently grey filters with total den-

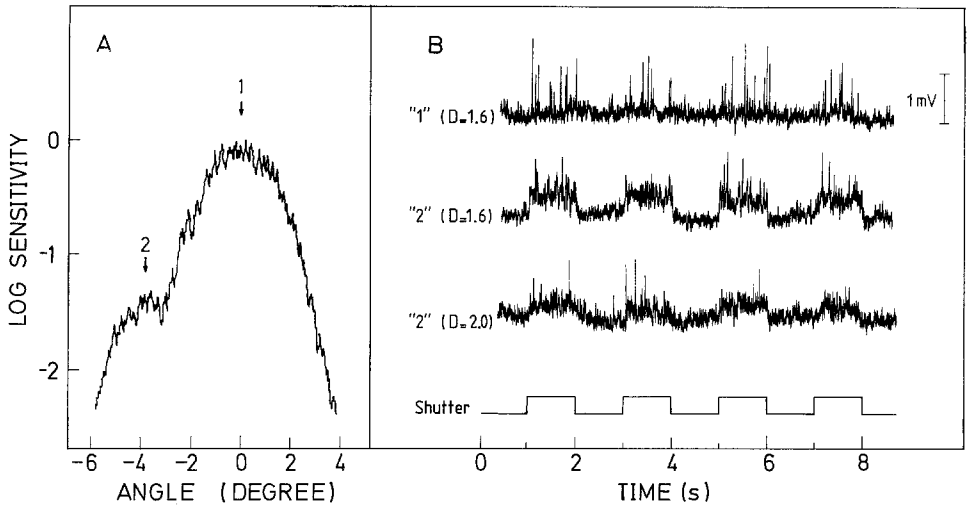

Fig. 3. A. Angular sensitivity of a R1–6 photoreceptor measured at a wavelength of 494 nm. The response of the cell is clamped by the feedback system at 3 mV. The arrows indicate the angular position of the stimulating beam for measuring quantum bumps. **B.** Recordings of quantum bumps after 10 min of dark adaptation. For measuring bumps grey filters with total density $D=1.6$ are added to the stimulating beam. On the top ('1') of the angular sensitivity curve only large bumps are recorded, while at the position of the sideband ('2') a small continuous signal is added to the bumps. The amplitude of this continuous signal depends on the light intensity; with grey filter $D=2$ the continuous signal is smaller than with $D=1.6$

sity D = 1.6 were added and dark adaptation was allowed for 10 min. After opening the shutter, bumps of about 1 mV were recorded. When the light beam was positioned at an asymmetrical sideband, and the light intensity was made as effective as in the former case, again bumps of about 1 mV were recorded (Fig. 3B). However, the number of these bumps was lower than that occurring at the peak of the angular sensitivity and a small continuous signal was added. This continuous signal is light-dependent (Fig. 3B, D = 2).

It is likely that this continuous signal originates from electrical coupling with a neighbouring cell because when the light beam is positioned at the sideband, it is also on-axis for a neighbouring cell. Then the penetrated cell is weakly stimulated and the neighbouring cell is strongly stimulated. Part of the large light-induced signal of the neighbouring cell probably leaks to the very weakly stimulated penetrated cell, so causing the small continuous signal in the recordings. It is likely that the electrical interactions are due to coupling with neighbouring R1–6 photoreceptors, because a typical R1–6 spectral sensitivity was always measured when the light beam was positioned on top of the sideband. The asymmetrical angular sensitivity profile thus originates from the outputs of different cells. The coupling observed here cannot be optical as this would cause l-bumps alone.

The above test was performed on a large number of cells with asymmetrical sidebands. The number of sidebands was variable, but in all recordings stimulation at the position of the sidebands resulted in bumps on top of a continuous signal. Some cells appeared to have electrical interactions with all surrounding cells while others only had electrical input from one neighbour. For reasons that will be discussed in the next section no attempt has been made to classify all these variable input configurations.

Origin of the electrical coupling

It is often supposed that simultaneous penetration of more than one cell with a glass microelectrode does not occur very frequently. Unfortunately, however, this assumption has never been systematically tested. During the measurements we noted that angular sensitivity profiles were broadened more often in the vertical than in the horizontal direction. The electrodes were lowered vertically and so we came to suspect the electrical coupling had an artificial origin. This suspicion was reinforced by an experiment in which the degree of electrical coupling was changed experimentally. In that particular case, without leaving the cell, the position of the electrode was moved over small distances. As can be seen in Fig. 4, the shape of the angular sensitivity profile varied strongly with the position of the electrode. Hence a change in the position of the electrode caused a change in the degree of the electrical coupling. We concluded

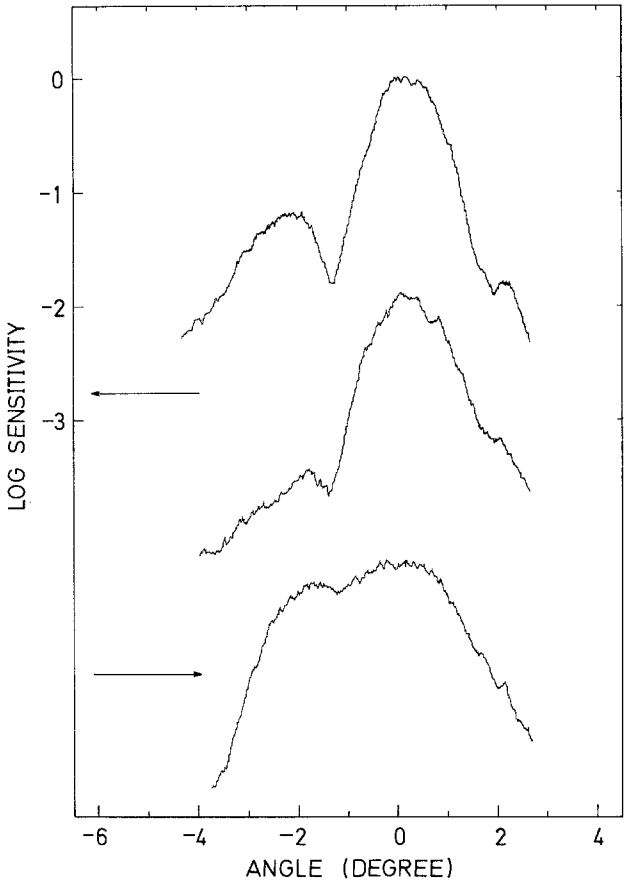

Fig. 4. Angular sensitivity of a R1–6 photoreceptor measured at a wavelength of 494 nm (upper curve). After the electrode is pulled slightly back the amplitude of the sideband is reduced (second curve). After a small advancement of the electrode the amplitude of the sideband is increased (third curve)

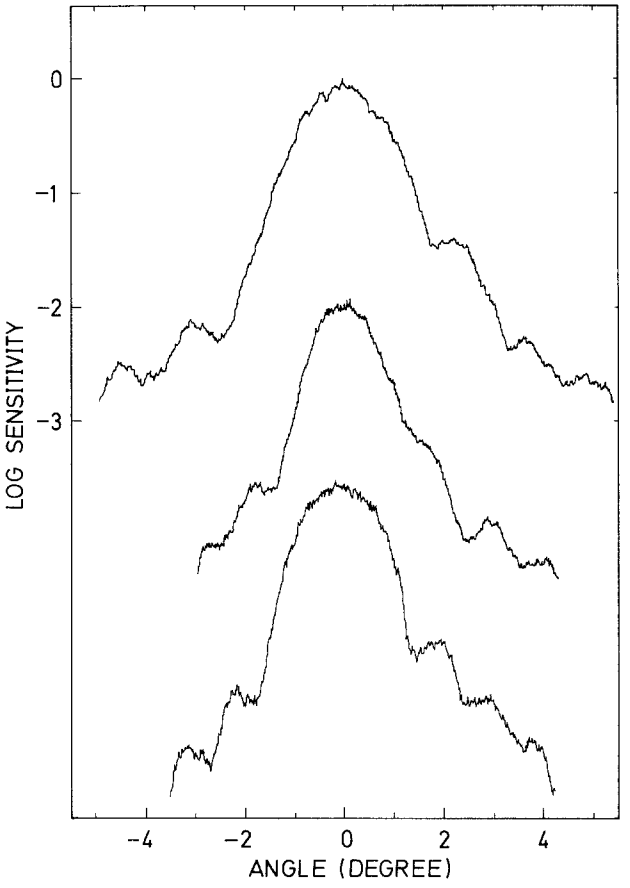

Fig. 5. As Fig. 1. Recordings from cells which are only slightly affected by electrical interactions. Both types of sidebands are present. The diffraction-like sidebands are elevated on one side due to artificial electrical coupling with a neighbouring cell

therefore that at least in this experiment, electrical coupling between the neighbouring cells could not be natural.

In order to test whether the degree of electrical coupling depends on electrode type we manufactured a series of electrodes with slightly different settings of the electrode puller. Although the electrodes hardly differed from each other in resistance or in appearance, as seen under the light microscope, the resulting recordings showed a large variation in angular sensitivity profiles. We therefore systematically changed the variables that could be set on the electrode puller, such as pull-strength, heat and several time constants, and used the amplitude of the recorded sidebands as a criterion to find the optimal setting of the electrode puller.

Early in this procedure all recordings showed cells that were evidently coupled, to a variable degree, with cells lying both in horizontal and vertical directions, within the same ommatidium of the penetrated cells (as in Fig. 1). Sometimes even more than one ommatidium was involved. After an initial period of testing and selecting electrodes only small electrical couplings remained (see Fig. 5). These mostly involved cells lying in the vertical plane, the plane of the electrode advancement.

At the end of this electrode selection procedure frequently cells were penetrated without any electrical coupling to neighbouring cells, or with only a small coupling with cells lying in the vertical plane. The tip of the electrodes made at the end of this selection procedure was somewhat longer and less tapered than that of the electrodes made at the start of this procedure. Distortion of the eye's optics by these electrodes seemed negligible and the diffraction pattern-like angular sensitivity profiles were measured. These profiles could be fully described by assuming an output of only one photoreceptor (Smakman et al. 1984). It is very unlikely that by a selection of electrodes a natural

electrical coupling can be lowered and so we conclude that at least some of the electrical couplings reported in the literature are artificial.

Discussion

Spatial sensitivity measurements were made to determine the nature of electrical coupling between neighbouring photoreceptor cells. We conclude that the observed electrical coupling is often artificial because:

1. The degree of electrical coupling was affected by slight movements of the microelectrode.
2. Electrical coupling was mostly observed in the direction of the electrode advancement rather than in a perpendicular direction.
3. A selection of electrodes could change the degree of electrical coupling.

The origin of artificial coupling is not clear. Possibly the electrodes penetrated more than one cell and/or pushed cells against each other, inducing connections between neighbouring cells at the site of penetration.

After the electrode selection procedure some broadening of angular sensitivity profiles was still found, perhaps due to the impossibility of producing electrodes which cause no electrical coupling at all. Another reason for small sidebands in the angular sensitivity profile are small distortions of the optics of the fly's eye. R1–6 cells of a neuro-ommatidium are electrically coupled (Shaw 1984; van Hateren 1986) and normally share the same visual axis. Defocussing can cause small sidebands in the angular sensitivity profile. The electrical signals from this type of electrical coupling are relatively small, however, so it is unlikely that this effect can cause the large sidebands reported here.

A phenomenon that might help the microelectrodes to induce artificial coupling is the spontaneous movement of the fly retina (see for example Kirschfeld and Franceschini 1969). Although these movements were minimized by the preparation technique (but not completely abolished, see Smakman et al. 1984), it is still likely that these continuous movements induce mechanical damage to the cell membrane around the microelectrode. This means that the sealing of the cell membrane to the microelectrode may never be perfect or that artificial electrical connections between neighbouring cells are almost inevitable. Nevertheless, as we have shown it is possible to measure from R1–6 photoreceptors which are not electrically coupled, we may hypothesize that there is no natural electrical coupling between R1–6 photoreceptors within one ommatidium. We note that optical coupling does occur but its contribution is minor (Wijngaard and Stavenga 1975).

Implications

Broadened angular sensitivity profiles are most probably due to experimental artefacts and so a few concepts have to be reconsidered. Firstly, the concept of the large receptive fields sampled by each R1–6 fly photoreceptor (Mimura 1978) is most probably false. Consequently, spatial summation of information does not take place in the first stage of information processing, the retina, but at a later one.

Recently Beersma (1979) and Dubs (1982) have presented angular sensitivity profiles with a large variation in shape. According to Dubs the angular sensitivity profiles measured by both authors are much alike, although the explanation of the variability is different. Beersma argued that the subsidiary peaks are caused by straylight, leaking through the secondary pigment cells between the photoreceptor cell and the adjacent ommatidium. Dubs interpreted part of the broadening of the angular sensitivity profile by assuming electrical coupling between adjacent photoreceptors. As has already been demonstrated, subsidiary peaks can originate from both the diffraction pattern of the facet lens and from artificial electrical coupling. As a result, a wide variability of subsidiary peaks can be measured with microelectrodes. It is our opinion, at present, that part of the large variability in the measurements mentioned above most probably had an artificial origin.

Considerable weight is added to this view by the recent findings of van Hateren (1986), demonstrating a close correspondence between angular sensitivity curves which were measured by optical and electrophysiological methods and which were theoretically predictable.

Interestingly, Howard (1983) in his thorough statistical study on bumps in locust photoreceptors was unable to distinguish l- and s-bumps, Lillywhite's two populations (1978). Howard thus concluded that electrical coupling was very low or non-existent in his experiments. Doujak (1984) reached a similar conclusion for crab photoreceptors. Dubs et al. (1981) provided evidence that s-bumps occur in fly LMC's under off-axis illumination. It is difficult to make a quantitative statement on these bumps because they are indistinguishable from the dark noise of the cell. As in the retinular cells, these s-bumps can only be demonstrated by an increase of the noise level during off-axis illumination. According to Dubs (1982) the s-bumps in

the LMC response may be the result of the transmission of s-bumps from the photoreceptors to the LMC. If this is true, the origin of the s-bumps in the photoreceptors is not artificial. However, alternative explanations are also possible, because it is just as likely that the s-bumps of the LMC's originate from interactions between lamina cells instead of retinular cells. Interactions between lamina cells may be natural, but if microelectrodes can induce coupling between photoreceptors, they may equally well be able to couple lamina cells. This possibility has to be accounted for in further investigations into the origin of the s-bumps in the lamina cells.

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